

### Mixed infections of *Chlamydia trachomatis* may be missed using nested PCR

Recently we reported our epidemiological findings for the serovar determination of *Chlamydia trachomatis* strains from Sheffield and Greater Manchester.<sup>1</sup> Strains were typed by detection of restriction fragment length polymorphism in the gene encoding the major outer membrane protein (MOMP). Although we were pleased to see that all 118 strains of *C. trachomatis* could be typed, we were aware that the DNA restriction patterns matched perfectly with known serovar control strains, and multiple serovars were not observed. Subsequently in a larger study of 141 strains (results not shown—to be published elsewhere) we were able to demonstrate the presence of multiple serovars in three samples. However, the findings of our initial study prompted us to investigate the parameters needed for multiple serovars to be detected using nested PCR.

The original PCR technique of Frost *et al.*<sup>2</sup> on which our nested PCR method was based was able to detect multiple serovars in a single sample, but only when present in stoichiometric quantities. Out of 119 patients a different PCR technique was able to detect two specimens (1.7%) which both contained two serovars; however, no details were given of the relative quantities of each serovar needed for detection.<sup>3</sup>

We decided to mix single control serovars, D, E and F kindly provided by Dr. Treharne, Institute of Ophthalmology, London. These serovars were selected as they were found to be the most commonly detected in our study<sup>1</sup>, which is in agreement with many such studies on serovar prevalence. Using the nested PCR method as previously described,<sup>1</sup> we titrated the control serovars to allow us to judge the relative amounts of chlamydial DNA present. By adjusting the concentrations of chlamydial DNA from the control serovars, mixtures were prepared containing approximately equal amounts of serovars D and E, D and F, and E and F. At the same time 10-fold less concentrated amounts of DNA were also prepared for each serovar and all possible mixtures of neat and diluted samples prepared. After the nested PCR technique had been applied to all mixtures, the amplified DNA was digested as before and separated on polyacrylamide gels.<sup>1</sup> In addition, three clinical strains were tested for each serovar in triplicate and findings compared with restriction endonuclease fragment patterns of control strains of the 15 serovars of *C. trachomatis*.

The results are presented in the table. When strains were mixed in equal amounts, all but the combination of D and E (which denoted a mixture) gave the appearance of serovar F. When undiluted samples containing a single serovar were mixed with another 10-fold less concentrated serovar, the resulting appearance was always that of the more concentrated serovar, except in the case of E and F (10:1), which indicated the presence of this mixture.

We agree in part, therefore, with the findings of Frost<sup>2</sup> in that multiple serovars can be detected in stoichiometric quantities (such as D and E). However, we found that serovar F dominated in equal mixtures with both D and E and could still be detected when present in a 10-fold less concentrated mixture with E.

We conclude that the determination of multiple serovars using the nested PCR technique is very dependent on the exact proportions of the strains in the clinical sample, and that in mixtures where one serovar is more concentrated, multiple serovars may not be detected. Although we believe that mixed chlamydia infections are quite rare, being found in approximately 2% of specimens,<sup>4</sup> we are uncertain of their clinical significance. Indeed, their exact prevalence is probably unknown as there are also disadvantages with the more conventional immunotyping method used, in that serial passages required for high titre preparations may select for growth of one serovar over another. Moreover, other workers have recently shown discrepancies between PCR and immunotyping methods for the detection of multiple serovars in five out of seven mixed infections.<sup>5</sup> This emphasises the need for a reliable method to determine multiple serovars of *C. trachomatis* so that we may gain more knowledge of their true clinical relevance and prevalence in genital disease.

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- 2 Frost EH, Deslandes S, Veilleux S, *et al.* Typing of *Chlamydia trachomatis* by detection of restriction fragment length polymorphism in the gene encoding the major outer membrane protein. *J Infect Dis* 1991;163:1103–7.
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Table Serovar determination of mixtures of *C. trachomatis* strains using nested PCR

Serovar mixtures	(ratio)	PCR determination of serovar(s)
D + E	(1:1)	D + E
D + F	(1:1)	F
E + F	(1:1)	F
D + F	(10:1)	D
D + E	(10:1)	D
E + D	(10:1)	E
E + F	(10:1)	E + F
F + D	(10:1)	F
F + E	(10:1)	F

### Positive syphilis test results in Down's syndrome in the past: a contemporary explanation

While carrying out some historical research, I was interested to discover that in the early

part of the 20th century children with Down's syndrome were tested for syphilis; it was considered that syphilitic infection in their parents through damage to the germ cells could have led to the production of offspring with this syndrome. I was even more interested to find, however, that in a couple of American studies<sup>1,2</sup> some positive Wassermann and Lange gold chloride test results were obtained and it was felt that it could be concluded "the tests prove beyond question that this condition [Down's syndrome] is a result of syphilitic infection".<sup>2</sup>

These were almost certainly false positive syphilis test results. Such results may occur in autoimmune disorders. In the Wassermann reaction, certain autoantibodies crossreact with cardiolipin, and it is, I think, relevant here that findings indicative of autoimmune haemolytic anaemia have been often found in Down's syndrome. Positive colloidal gold chloride test results may be obtained in immune-mediated neuropathies as a result of elevation of CSF protein, and pertinent findings in Down's syndrome include demyelination in brain and spinal cord, sensory and motor disturbances, and intellectual impairment.

Autoantibodies have been detected quite frequently in people with Down's syndrome as part of a study of their thyroid glands, and I believe that the possibility needs to be considered that a great many of the disorders of Down's syndrome are autoimmune in origin. Fibroblasts trisomic for chromosome 21 show a significantly enhanced response to the antiviral effects of both type I and type II interferon,<sup>3</sup> and IFN-gamma has been found to be a very good in vitro stimulus for the induction of HLA-DR expression in a number of endocrine tissues, including thyroid epithelium.<sup>4</sup> It has been hypothesised previously that IFN-gamma production by normal blood lymphocytes is at too low a concentration in vivo to allow Ia antigen expression by normal human thyroid cells.<sup>5</sup> It is possible that the increased sensitivity of Down's syndrome cells to type I and type II interferon could lead to thyroid cells (and potentially many other cells, as well as the endocrine) expressing autoantigens and, subsequently, autoimmune disease developing.

Thus, rather than Down's syndrome being a result of syphilis as postulated in 1916, I believe that it should be added to the list of disorders which, through an autoimmune mechanism, can give rise to biological false positive tests for syphilis.

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### Penicillinase producing *Neisseria gonorrhoeae* associated with severe vulvovaginitis in a post menopausal woman

I was interested to note the case report from Bodsworth *et al*<sup>1</sup> of gonococcal mastitis in a male and the detailed study of penicillinase producing *Neisseria gonorrhoeae* (PPNG) from Warren and Phillips.<sup>2</sup> I would like to report a further atypical presentation of gonococcal infection in which a PPNG was isolated.

A 54 year old patient attended the department of GenitoUrinary Medicine in Swansea in August 1992 following referral from her general practitioner. She complained of malodorous vaginal discharge, superficial dysuria and severe vulval soreness. Symptoms had started about 24 hours after a casual unprotected sexual encounter whilst on holiday on a Greek island some two weeks previously. She had had a hysterectomy for menorrhagia 10 years before and was not taking hormone replacement therapy. Previous intercourse had occurred over eighteen months prior to the single sexual encounter which led to her attendance.

On examination of the vulva, a putrid odour was apparent and the vulval mucosa was uniformly red, swollen and covered with sticky yellow secretions. The appearance and texture of the vulva was that of recently cut silverside of beef. No vulval or perianal fissures or urethral discharge were noted and on passing a vaginal speculum a copious viscous yellow discharge coated the vaginal walls. The vaginal mucosa was uniformly red but less swollen than the vulva. The cervix was absent. There was no inguinal lymphadenopathy and no systemic symptoms or signs of infection. High vaginal swabs were taken for microscopy and culture for *Neisseria gonorrhoea*, *Trichomonas vaginalis* and *Candida* species. An ELISA test (Novo Nordisk) for *Chlamydia trachomatis* was also performed on the vaginal secretions. Urethral swabs were taken for microscopy and culture of *N gonorrhoeae*.

Examination of a wet-mount preparation of vaginal secretions was negative for trichomonads and fungal elements but masses of polymorphonuclear leucocytes (PMNLs) were noted. A Gram-stained preparation of the secretions revealed confluent PNMLs the majority of which contained Gram negative intracellular diplococci morphologically identical to *Neisseria gonorrhoeae*. Scattered parabasal/intermediate cells and a scanty mixed anaerobic vaginosis type bacterial flora<sup>3</sup> was also noted. The urethral swab revealed occasional PNMLs but no intracellular diplococci. A provisional diagnosis of gonococcal infection associated with an